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Studies on the microflora of Danish beech forest soils

III. Properties and composition of the bacterial flora

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The object of the investigations recorded in this paper, was to study the bacterial types occurring in beech mull and beech mor, and their relative incidence, in order to test whether a significant difference in composition of the bacterial flora of the two soil types could be established in this way.

The material for the study consists in transfers from different series of plate counts, described in detail in a previous paper of this series (JENSEN 1963), which also contains detailed descriptions of the localities investigated.

In investigations of this type, it is necessary to work with relatively large numbers of strains, and therefore it is impracticable to make a complete identification of each individual strain. Instead, some sort of classification must be used, and this is usually made on the basis of morphology, biochemical activities or nutritional requirements.

In the investigations recorded here, the classification in botanical groups based on morphological criteria has been given a preferential position, partly because this method seems to show existing differences most clearly, and partly because it was the intention that the classification should be succeeded by more detailed taxonomic studies.

Qualitative studies on the soil microflora on the basis of morphology have been carried out, using rather different systems of classification (see e. g. SNOW 1935, LOCHHEAD and CHASE 1943, WEBLEY et al. 1952, VÁGNEROVÁ et al. 1960). The main difficulty in work of this type is the inevitable occurrence of transitional strains, which with equal right can be classed with two neighbouring groups.

In the system used here, the bacterial strains were arranged in the following 7 classes:

1. Gram-negative, non-pigmented rods.
2. Gram-negative, pigmented rods.
3. Sporeforming rods.
4. Gram-positive, pleomorphic rods.
5. Gram-negative, pleomorphic rods.
6. Streptomycetes.
7. Others.

The allocation of the Gram-negative rods to group 1 and 2 were made in such a way that group 2 only received strains with a clearly visible pigmentation. All doubtful cases, and all strains with fluorescent pigmentation only, were classed with group 1, because pigmentation of this nature is highly dependent on growth conditions and often not observable on the soil extract medium used for the transfers.

The group of sporeforming rods is in itself a well-defined group, but extremely sparsely sporulating strains do occur, and it is possible that a certain number of such strains have escaped the attention of the observer, as only a limited time can be spent on the examination of each strain.

The line of demarcation between the regular rods and the next two groups, the pleomorphic rods, is very indistinct and depends wholly on the judgment of the observer. Also the Gram-reaction of the pleomorphic rods can raise doubts in many cases, because all shades of Gram-positiveness are found within this group. Here, most of the Gram-variable strains were included in group 4, so that the Gram-negative group included only purely Gram-negative strains and strains only very slightly Gram-positive.

Group no. 6, comprising the streptomycetes, is the most easily recognizable of all the groups. Strains belonging to this group very seldom give rise to doubts.

The investigations fall naturally in three series, and in the following more details of the technique and descriptions of the other classification methods used, are given in connection with each of the series.

Series 1

The first series comprises transfers from plate counts on 6 soil samples, drawn on November 10th 1955 (see table 1). The counting medium was the mannitol asparagine salts agar of THORNTON (1922). After counting, transfers were made from a number of colonies to Bacto nutrient agar in culture tubes. In order to secure a correct representation of the different groups of bacteria, transfers were made from all colonies on a plate or on a definite sector of a plate, omitting only distinctly mixed or contaminated colonies.

The cultures were incubated for 1—2 days at 25° C; a brief description was then made of each culture, comprising pigmentation and macroscopical appearance of the agar stroke, and the microscopical appearance of unstained and Gram-stained preparations. On the basis of these descriptions the cultures were classified in the seven morphological groups mentioned above (see table 2).

Later a few biochemical tests were made on the same strains.

Production of acid from glucose was tested by means of nutrient agar containing 1 per cent glucose and phenol red as indicator. Hydrolysis of gelatin was tested on Bacto nutrient gelatin, and hydrolysis of starch on Bacto nutrient agar with 1 per cent soluble starch. Finally nitrate reduction was tested on Bacto nutrient agar containing 0.1 per cent KNO_3, using Griess' reagent for detection of nitrite.

The results are recorded in table 3.

The results from series 1 indicate that the pigmented, Gram-negative rods, the pleomorphic rods and the streptomycetes are more frequent in mull than

in mor soils, whereas the sporeforming rods are more numerous in the mor soils, even when the absolute numbers per gm. of dry soil are calculated.

The general impression after the examination of the cultures was that the bacterial flora of the mor soils was composed of comparatively few types, whereas a much larger variation was encountered in the mull soils.

As regards the biochemical activity, however, no definite difference could be established between the two soil types.

Table 1
Survey of soil samples
Series 1

Soil type	Locality	Date of sampling	pH	Humidity % of d. m.	Plate counts on Thornton's agar mill. per gm. d. m.
Beech	I	10. 11. 1955	5.3	54	11.1
mull	III	—	5.2	70	12.9
	VI	—	5.7	52	7.8
Beech	II	10. 11. 1955	4.1	104	1.5
mor	IV	—	4.4	117	2.2
	V	—	4.5	133	1.9

Table 2
Distribution to morphological groups
Series 1

Locality	Total number of transfers	No growth	Gram-neg. non-pigm. rods	Gram-neg. pigm. rods	Spore-forming rods	Gram-pos. pleomorph. rods	Gram-neg. pleomorph. rods	Streptomycetes	Others
mull soils	77	8	43	5	0	4	2	13	2
I	104	17	42	10	1	9	0	24	1
III	117	25	57	6	2	4	5	18	0
VI									
total	298	50	142	21	3	17	7	55	3
percentage	100.0	16.8	47.7	7.1	1.0	5.7	2.3	18.4	1.0
mor soils	50	12	23	0	12	0	0	3	0
II	75	13	55	1	5	1	0	0	0
IV	54	12	31	1	1	2	0	7	0
V									
total	179	37	109	2	18	3	0	10	0
percentage	100.0	20.7	60.9	1.1	10.0	1.7	0.0	5.6	0.0

Table 3
Biochemical activity within morphological groups
Series I

Group	Number of strains	Acid from glucose		Hydrolysis of gelatin		Hydrolysis of starch		Reduction of nitrate	
		total	%	total	%	total	%	total	%
Gram-neg., non-pigm. rods	134	73	54.5	60	44.8	8	6.0	42	31.3
Gram-neg., pigm. rods	21	5	23.8	18	85.7	14	66.7	9	42.9
Sporeforming rods	3	3	100.0	1	33.3	2	66.7	1	33.3
Pleomorph. Gram-pos. rods	17	1	5.9	3	17.6	1	5.9	1	5.9
Pleomorph Gram-neg. rods	5	0	0.0	1	20.0	1	20.0	0	0.0
Streptomycetes	51	9	17.6	48	96.1	42	82.4	21	41.2
Others	3	1	33.3	1	33.3	0	0.0	0	0.0
Mull, total	234	92	39.3	132	56.4	68	29.1	74	31.6
Gram-neg., non-pigm. rods	105	42	40.0	55	52.4	3	2.9	35	33.3
Gram-neg., pigm. rods	2	1	50.0	2	100.0	1	50.0	1	50.0
Sporeforming rods	18	13	72.2	11	61.1	9	50.0	8	44.4
Pleomorph. Gram-pos. rods	3	2	66.7	3	100.0	0	0.0	0	0.0
Pleomorph. Gram-neg. rods	0	—	—	—	—	—	—	—	—
Streptomycetes	10	0	0.0	10	100.0	10	100.0	3	30.0
Others	0	—	—	—	—	—	—	—	—
Mor, total	138	58	42.0	81	58.7	23	16.7	47	34.1

Series II

Series II originates in plate counts on 6 soil samples, drawn in April and and May 1956 from the same 6 localities as in series I, and on 4 soil samples, drawn in April 1957 from 4 new localities (see table 4). In this series, platings were made on three different media: Thornton's agar, Bacto tryptone glucose extract agar, and soil extract agar. Transfers were made from about 100 colonies on each of these media.

In series I a rather large percentage of transfers failed to develop, and therefore another medium was used for the transfers both in this and in the following series, viz. soil extract agar with addition of 0.1 per cent glucose and 0.01 per cent yeast extract.

Examination and classification in morphological groups were made as in series I (see tables 5 and 6).

The results from series II confirm the results from series I as regards the relative incidence of Gram-negative, pigmented rods and Gram-positive, pleo-

morphic rods, both types being much more common in mull than in mor soils. As to the sporeforming rods and the streptomycetes, however, the results are reversed, but the general impression of a richer and more varied bacterial flora in mull than in mor is sustained.

When the three media are compared, the most striking difference is found in the percentage incidence of sporeforming rods, i. e. on an average 4.0 per cent on Thornton's agar, 9.6 per cent on tryptone glucose extract agar, and 22.2 per cent on soil extract agar.

Table 4
Survey of soil samples
Series II

Soil type	Locality	Date of sampling	pH	Humidity % of d. m.	Plate counts, mill. per gm. of d. m.		
					Thornton's agar	Tryptone glucose extract agar	Soil extract agar
Beech mull	I	13. 4. 56	5.1	45	9.5	5.1	7.2
	III	3. 5. 56	—	54	3.5	2.4	3.9
	VI	3. 5. 56	6.9	39	20.6	7.2	8.7
	VII	6. 4. 57	5.4	37	5.4	11.2	5.6
	X	6. 4. 57	4.7	56	0.8	2.0	1.2
Beech mor	II	13. 4. 56	4.5	104	4.4	13.1	2.9
	IV	3. 5. 56	4.7	104	3.6	7.0	4.0
	V	3. 5. 56	—	117	7.5	7.5	8.4
	VIII	6. 4. 57	4.4	163	1.8	4.7	2.0
	IX	6. 4. 57	4.3	213	3.1	15.1	4.3

Series III

This series comprises transfers from plate counts on 6 samples of surface soil and 4 samples of mineral soil (35 cm below surface) drawn in May, July and December 1958, from the same 6 localities as in series I. Furthermore for comparison, examination was made of 2 samples drawn from the following localities:

XI. A spruce stand, c. 50 years old, with a typical mor soil, almost devoid of vegetation apart from a few light spots, where the ground was covered with *Majanthemum bifolium*, *Oxalis acetosella* and *Aira flexuosa*. pH 4.1, organic matter content 58 per cent, C/N ratio 28.

XII. A spruce stand, c. 50 years old and very similar to the preceding, with a typical mor soil, and a sparse ground vegetation consisting of various mosses and scattered tufts of *Aira flexuosa*. pH 4.2, organic matter content 57 per cent, C/N ratio 31.

This time the plate counts were made exclusively on soil extract agar, and the examinations include both a morphological classification as in the two preceding series (see table 8), and a nutritional classification made according to the methods of LOCHHEAD and CHASE (1943) (see tables 10 and 11).

As regards the morphological classification, series III, in connection with the preceding series (see table 9), shows that consistent differences between mull

Table 5

Distribution to morphological groups
Mull soils, series II

Counting medium	Locality	Number of transfers	No growth	Gram-neg. non-pigm. rods	Gram-neg. pigm. rods	Spore-forming rods	Pleomorph. Gram-pos. rods	Pleomorph. Gram-neg. rods	Strepto-mycetes	Others
Thornton's agar	I	100	17	38	12	5	2	2	23	1
	III	100	20	31	14	4	12	4	14	1
	VI	100	13	20	13	0	21	6	21	6
	VII	117	5	56	10	5	13	3	22	3
	X	49	21	10	0	4	1	1	10	2
	total	466	76	155	49	18	49	16	90	13
	percentage	—	16.3	33.3	10.5	3.9	10.5	3.4	19.3	2.8
Tryptone glucose extract agar	I	100	16	39	5	1	5	0	33	1
	III	100	12	18	6	14	7	0	36	7
	VI	100	2	21	15	6	24	1	29	2
	VII	100	3	46	14	10	9	4	10	4
	X	100	18	23	2	38	2	0	12	5
	total	500	51	147	42	69	47	5	120	19
	percentage	—	10.2	29.4	8.4	13.8	9.4	1.0	24.0	3.8
Soil extract agar	I	100	8	37	14	5	12	0	22	2
	III	100	4	26	13	20	14	7	14	2
	VI	100	5	22	16	7	14	4	30	2
	VII	121	3	28	17	34	18	1	13	7
	X	66	3	7	0	46	2	0	6	2
	total	487	23	120	60	112	60	12	85	15
	percentage	—	4.7	24.6	12.3	23.0	12.3	2.5	17.5	3.1
All three media	total	1453	150	422	151	199	156	33	295	47
	percentage	—	10.3	29.2	10.4	13.7	10.7	2.3	20.3	3.2

Table 6

Distribution to morphological groups
Mor soils, series II

Counting medium	Locality	Number of transfers	No growth	Gram-neg. non-pigm. rods	Gram-neg. pigm. rods	Spore-forming rods	Pleomorph. Gram-pos. rods	Pleomorph. Gram-neg. rods	Strepto-mycetes	Others
Thornton's agar	II	100	26	55	0	6	2	0	11	0
	IV	100	6	45	0	8	0	1	37	3
	V	100	5	49	10	4	5	4	20	3
	VIII	53	11	12	0	1	1	0	24	4
	IX	102	9	27	0	0	1	2	59	4
	total	455	57	188	10	19	9	7	151	14
	percentage	—	12.5	41.3	2.2	4.2	2.0	1.5	33.2	3.1
Tryptone glucose extract agar	II	100	34	52	1	2	1	0	8	2
	IV	100	9	40	5	8	0	1	29	8
	V	100	12	45	5	1	6	0	22	9
	VIII	100	30	27	0	7	0	0	26	10
	IX	100	17	35	0	9	3	2	19	15
	total	500	102	199	11	27	10	3	104	44
	percentage	—	20.4	39.8	2.2	5.4	2.0	0.6	20.8	8.8
Soil extract agar	II	100	18	31	0	34	1	0	14	2
	IV	100	4	10	5	22	2	1	43	13
	V	100	1	33	22	8	8	3	18	7
	VIII	63	2	5	0	24	3	0	28	1
	IX	116	10	23	0	15	9	3	44	12
	total	479	35	102	27	103	23	7	147	35
	percentage	—	7.3	21.3	5.6	21.5	4.8	1.5	30.7	7.3
All three media	total	1434	194	489	48	149	42	17	402	93
	percentage	—	13.5	34.1	3.3	10.4	2.9	1.2	28.0	6.5

and mor soils are found mainly in the frequency of the minor groups: the Gram-negative, pigmented rods, and the Gram-positive and Gram-negative, pleomorphic rods. All three groups were much more numerous in mull than in mor soils.

The largest group both in beech mull and in beech mor is the Gram-negative, non-pigmented rods, and when the average numbers are considered, no difference is found between the two soil types as regards this group. The next largest group is the streptomycetes. In this group the average numbers indicate a greater frequency in mor than in mull soils. However, this may be incidental, as the number of streptomycetes varies very much from time to time, and seems to be more dependent on other factors, especially humidity, than on the soil type. The number of sporeforming rods, the third largest group, is also very variable, and the average numbers do not indicate consistent differences between mull and mor.

Table 7
Survey of soil samples
Series III

Soil type	Locality	Date of sampling	pH	Humidity % of d. m.	Plate counts on soil extract agar mill. per gm. d. m.
Beech mull	I	3. 7. 58	5.4	25	10.3
	III	2. 12. 58	4.7	43	2.5
	VI	5. 5. 58	5.3	52	16.6
Beech mor	II	3. 7. 58	3.8	113	8.2
	IV	2. 12. 58	4.2	144	5.1
	V	5. 5. 58	4.5	122	25.9
Beech mineral soils	I	3. 7. 58	4.8	12	0.5
	II	3. 7. 58	4.8	14	0.2
	III	2. 12. 58	4.9	25	1.1
	IV	2. 12. 58	5.0	15	0.5
Spruce mor	XI	3. 7. 58	4.1	92	6.8
	XII	2. 12. 58	4.2	144	2.1

The four mineral soils show a percentage distribution very different from that found in the surface soils, the bacterial flora consisting almost exclusively in Gram-negative, non-pigmented rods and sporeforming rods. The distinct difference between locality III and the remaining localities is due to a deeper A-horizon in locality III. The four samples were drawn from the same depth below surface, and the sample from locality III is more affected by the surface soil than the other three samples, which appeared to be purely mineral (cf. V. JENSEN 1962).

The spruce mor showed a clear relationship to the beech mor; the Gram-negative, pigmented rods were lacking and the pleomorphic rods very few in number. The remaining groups fall within the same range as in the other surface soils.

Table 8

Distribution to morphological groups
Series III

Locality	Number of transfers	No growth	Gram-neg. non-pigm. rods	Gram-neg. pigm. rods	Spore-forming rods	Pleomorph. Gram-pos. rods	Pleomorph. Gram-neg. rods	Strepto-mycetes	Others
Beech mull									
I	100	2	18	18	33	7	2	18	2
III	100	5	42	0	23	10	1	16	3
VI	100	1	35	7	20	11	3	21	2
total	300	8	95	25	76	28	6	55	7
percentage	—	2.7	31.7	8.3	25.4	9.3	2.0	18.3	2.3
Beech mor									
II	100	0	6	2	78	2	2	8	2
IV	98	7	15	0	61	2	1	11	1
V	110	5	15	0	11	0	0	77	2
total	308	12	36	2	150	4	3	96	5
percentage	—	3.9	11.7	0.6	48.7	1.3	1.0	31.2	1.6
Beech sub-soils									
I	97	2	86	1	1	0	7	0	0
II	96	0	76	0	20	0	0	0	0
III	99	3	4	0	79	5	1	4	3
IV	100	1	82	0	5	4	3	3	2
total	392	6	248	1	105	9	11	7	5
percentage	—	1.5	63.2	0.3	26.8	2.3	2.8	1.8	1.3
Spruce mor									
XI	100	5	9	0	26	4	1	51	4
XII	100	1	36	0	53	3	0	2	5
total	200	6	45	0	79	7	1	53	9
percentage	—	3.0	22.5	0.0	39.5	3.5	0.5	26.5	4.5

Table 9

Summary of the morphological classification
(Percentage distribution of the isolated strains)

Locality	Total number of transfers	No growth	Gram-neg. non-pigm. rods	Gram-neg. pigm. rods	Spore-forming rods	Pleomorph. Gram-pos. rods	Pleomorph. Gram-neg. rods	Strepto-mycetes	Others
Beech mull									
Series I	298	16.8	47.7	7.1	1.0	5.7	2.3	18.4	1.0
,, II	1453	10.3	29.2	10.4	13.7	10.7	2.3	20.3	3.2
,, III	300	2.7	31.7	8.3	25.4	9.3	2.0	18.3	2.3
total	2051	10.1	32.2	9.6	13.6	9.8	2.3	19.7	2.7
Beech mor									
Series I	179	20.7	60.9	1.1	10.0	1.7	0.0	5.6	0.0
,, II	1434	13.5	34.1	3.3	10.4	2.9	1.2	28.0	6.5
,, III	308	3.9	11.7	0.6	48.7	1.3	1.0	31.2	1.6
total	1921	12.7	33.0	2.7	16.5	2.6	1.0	26.4	5.1
Beech mineral soils	392	1.5	63.2	0.3	26.8	2.3	2.8	1.8	1.3
Spruce mor	200	3.0	22.5	0.0	39.5	3.5	0.5	26.5	4.5

Table 10

Distribution to nutritional groups
Series III
(Explanation in text p. 605)

Locality	Number of transfers	No growth	Nutritional groups							
			I	II a	II b	III	IV	V	VI	VII
Beech mull										
I	97	1	12	17	8	9	2	41	3	4
III	95	0	30	24	8	6	2	20	1	4
VI	98	5	8	39		2	7	32	2	3
total	290	6	50	96		17	11	93	6	11
percentage	—	2.1	17.2	33.1		5.9	3.8	32.0	2.1	3.8
Beech mor										
II	100	6	7	14	0	13	17	32	5	6
IV	90	5	12	17	13	11	5	21	1	5
V	105	1	5	47		1	5	43	2	1
total	295	12	24	91		25	27	96	8	12
percentage	—	4.1	8.3	30.8		8.4	9.1	32.5	2.7	4.1
Beech mineral soils										
I	96	0	89	4	2	0	0	1	0	0
II	97	2	61	20	3	4	3	4	0	0
III	95	3	4	8	17	10	15	27	2	9
IV	99	0	77	14	3	0	1	4	0	0
total	387	5	231	46	25	14	19	36	2	9
percentage	—	1.3	59.7	11.9	6.5	3.6	4.9	9.3	0.5	2.3

Table 11
Distribution to nutritional groups within the morphological groups
Series III

Soil type	Nutritional group	Total number of transfers		Gram-neg. rods non-pigmented		Gram-neg. rods pigmented		Spore-forming rods		Pleomorphic Gram-pos. rods		Pleomorphic Gram-neg. rods		Streptomycetes		Others	
		total	%	total	%	total	%	total	%	total	%	total	%	total	%	total	%
Beech mull	I	50	17.2	29	30.5	1	4.0	5	6.8	6	20.7	0	0.0	6	10.9	3	42.8
	II a + b	96	33.1	26	27.3	3	12.0	14	18.9	15	51.8	1	20.0	34	61.8	3	42.8
	III	17	5.9	6	6.3	0	0.0	9	12.2	0	0.0	0	0.0	1	1.8	1	14.4
	IV	11	3.8	4	4.2	0	0.0	4	5.4	2	6.9	1	20.0	0	0.0	0	0.0
	V	93	32.0	22	23.2	17	68.0	33	44.6	5	17.2	3	60.0	13	23.7	0	0.0
	VI	6	2.1	3	3.2	2	8.0	1	1.3	0	0.0	0	0.0	0	0.0	0	0.0
	VII	11	3.8	2	2.1	0	0.0	8	10.8	1	3.4	0	0.0	0	0.0	0	0.0
	no growth	6	2.1	3	3.2	2	8.0	0	0.0	0	0.0	0	0.0	1	1.8	0	0.0
	total	290	—	95	—	25	—	74	—	29	—	5	—	55	—	7	—
Beech mor	I	24	8.3	6	15.8	0	0.0	10	6.8	1	33.3	0	0.0	2	2.1	5	83.3
	II a + b	91	30.8	18	47.4	1	50.0	24	16.3	0	0.0	1	33.3	47	49.0	0	0.0
	III	25	8.4	5	13.1	0	0.0	18	12.2	0	0.0	0	0.0	1	1.0	1	16.7
	IV	27	9.1	3	7.9	0	0.0	21	14.3	1	33.3	0	0.0	2	2.1	0	0.0
	V	96	32.5	3	7.9	1	50.0	48	32.7	1	33.4	2	66.7	41	42.7	0	0.0
	VI	8	2.7	1	2.6	0	0.0	5	3.4	0	0.0	0	0.0	2	2.1	0	0.0
	VII	12	4.1	0	0.0	0	0.0	11	7.5	0	0.0	0	0.0	1	1.0	0	0.0
	no growth	12	4.1	2	5.3	0	0.0	10	6.8	0	0.0	0	0.0	0	0.0	0	0.0
	total	295	—	38	—	2	—	147	—	3	—	3	—	96	—	6	—
Beech mineral soils	I	231	59.7	219	84.0	1	100.0	5	4.9	0	0.0	2	66.7	0	0.0	4	57.1
	II a + b	71	18.4	38	14.5	0	0.0	26	25.5	2	33.3	0	0.0	5	71.4	0	0.0
	III	14	3.6	2	0.7	0	0.0	11	10.8	1	16.7	0	0.0	0	0.0	0	0.0
	IV	19	4.9	1	0.4	0	0.0	17	16.7	0	0.0	0	0.0	0	0.0	1	14.3
	V	36	9.3	1	0.4	0	0.0	30	29.5	3	50.0	0	0.0	0	0.0	2	28.6
	VI	2	0.5	0	0.0	0	0.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0
	VII	9	2.3	0	0.0	0	0.0	7	6.7	0	0.0	1	33.3	1	14.3	0	0.0
	no growth	5	1.3	0	0.0	0	0.0	4	3.9	0	0.0	0	0.0	1	14.3	0	0.0
	total	387	—	261	—	1	—	102	—	6	—	3	—	7	—	7	—

The results of the nutritional classification are shown in tables 10 and 11. The nutritional groups are characterized as follows (cf. LOCHHEAD and CHASE 1943):

Group I: organisms showing good development in a mineral medium with glucose as carbon source and nitrate as sole source of nitrogen.
Group II: organisms requiring amino nitrogen. In most experiments this group was divided into:
Group II a: organisms growing well with ammonia as nitrogen source.
Group II b: organisms requiring one or more amino acids.
Group III: organisms, which require one or more growth factors. (B group of vitamins.)
Group IV: organisms requiring both amino acids and growth factors.
Group V: organisms requiring unidentified substances in yeast extract.
Group VI: organisms requiring unidentified substances in soil extract.
Group VII: organisms, which require unidentified substances in both yeast extract and soil extract.

The percentage distribution on the whole shows no significant difference between beech mull and beech mor, but for both soil types the distribution differs distinctly from that found normally in cultivated soils, especially in the very sparse occurrence of organisms belonging to group VII (see e. g. LOCHHEAD and CHASE 1943, LOCHHEAD and THEXTON 1947, STEVENSON and ROUATT 1953).

As regards the mineral soils, locality III does not differ much from the surface soils, but for the three remaining samples the great majority of the organisms belong to group I, the group with the most simple nutritional requirements.

In table 11 a survey is given of the distribution to nutritional groups within each of the morphological groups. As regards the Gram-negative, non-pigmented rods the table indicates a clear difference between the three soil types. For the mull soils c. 80 per cent of the strains are distributed about equally to the groups I, II and V. For the mor soils c. 75 per cent are distributed to the groups I, II and III with the majority in group II, and for the mineral soils 84 per cent are found in group I alone.

For the remaining morphological groups no such differences can be established. The greatest variation in nutritional demands is found among the sporeforming bacteria, which are distributed with the majority in the groups II, III, IV and V. The streptomycetes are found preferably in the groups II and V.

Taxonomic composition of the individual morphological groups

In order to obtain an idea of the genera and species constituting the different morphological groups a representative selection of strains was subjected to a closer taxonomic examination, the results of which may be summarized briefly as follows:

Gram-negative, non-pigmented rods

From this group 92 strains have been examined. 71 strains were tentatively identified as *Pseudomonas* spp., 16 strains as *Achromobacter* spp. and 5 strains as belonging to other genera. The predominance of the genera *Pseudomonas* and *Achromobacter* within this group of soil bacteria is in good agreement with the results found by previous investigators (e. g. STOUT 1958, 1960, HOLDING 1960).

The *Pseudomonas* strains, all actively motile, could be divided into the following 5 groups, mainly on the basis of occurrence of fluorescent pigmentation and fermentation of carbohydrates:

Group I: Fluorescent pigmentation; glucose fermented with formation of acid; gelatin is hydrolyzed; no hydrolysis of starch; nitrate is not reduced; litmus milk alkaline; no growth at 37° C.

Group II: Fluorescent pigmentation; glucose not fermented; hydrolysis of gelatin variable; no hydrolysis of starch; reduction of nitrate variable; litmus milk alkaline; no growth at 37° C.

Group III: No fluorescent pigmentation; glucose, but not lactose, fermented with formation of acid and gas; gelatin is hydrolyzed; no hydrolysis of starch; nitrate is reduced to nitrite; litmus milk alkaline; slight or no growth at 37° C.

Group IV: No fluorescent pigmentation; glucose fermented with formation of acid; hydrolysis of gelatin variable; no hydrolysis of starch; reduction of nitrate variable; litmus milk alkaline; no growth at 37° C.

Group V: No fluorescent pigmentation; glucose not fermented; hydrolysis of gelatin variable; no hydrolysis of starch; reduction of nitrate variable; litmus milk alkaline; no growth at 37° C.

The distribution of the strains to the 5 groups with reference to origin in mull or mor soils is tabulated in table 12. It appears that group V is the predominant group in mull soils and group I in mor soils.

Table 12

Distribution of 71 *Pseudomonas* strains to groups I—V

	Number of strains				
	I	II	III	IV	V
Mull soils	7	6	3	4	17
Mor soils	15	3	7	3	6

Of the 16 *Achromobacter* strains, 12 were isolated from mull soils and only 4 from mor soils. They constitute a more heterogeneous group than the pseudomonads, and no attempts have been made to classify them further.

Gram-negative, pigmented rods

Of this rather sparsely occurring group 18 strains have been examined, and 16 of these were classified as *Flavobacterium* spp. and 2 as *Chromobacterium* spp. The flavobacteria apparently belonged to at least 6 different species. The most common type (5 strains) seems to be closely related to *Flavobacterium arborescens* Bergey et al., but the examination was not sufficiently detailed to prove the identity.

Chromobacteria with violet pigmentation have been found now and again during the investigations. However, they are uncommon and occur almost exclusively in the mull soils.

Sporeforming rods

According to SMITH et al. (1952) the aerobic, sporeforming bacteria can be classified in four morphological groups, mainly on the basis of the appearance of spores and sporangia (see head of table 13). Generally, strains belonging to groups I and IV are easily recognizable, but in some cases it may be difficult to decide, whether the sporangia are „definitely" swollen or not, which make the boundary between groups II and III indistinct.

Table 13

Percentage distribution of c. 400 strains of sporeforming bacteria

	Sporangia not definitely swollen		Sporangia definitely swollen	
	Spores ellipsoidal		Spores ellipsoidal	Spores spherical
	diam. $> 0.9\ \mu$	diam. $< 0.9\ \mu$		
Group no.	I	II	III	IV
Mull soils, per cent. .	28.1	22.2	39.3	10.4
Mor soils, per cent . .	25.6	29.8	30.6	14.0

The results tabulated in table 13 show the percentage distribution of c. 400 strains of sporeforming bacteria, originating in the experimental series II and III. All four groups of sporeformers are represented abundantly in both mull and mor soils, and in this respect the results do not indicate any significant difference between the two soil types.

Table 14

Species distribution of 178 strains of *Bacillus* spp.

	Number of strains from		
	beech mull	beech mor	beech mineral soils
Bacillus megaterium	0	1	0
.. *cereus*	20	31	4
.. *cereus* var. *mycoides* .	2	4	0
.. *subtilis*	2	0	1
.. *pumilus*	1	3	0
.. *coagulans*	1	3	0
.. *firmus*	0	1	0
.. *lentus*	0	0	1
.. *polymyxa*	0	2	0
.. *circulans*	4	6	0
.. *laterosporus*	1	3	1
.. *pulvifaciens*	0	0	1
.. *pantothenticus*	0	1	0
.. *sphaericus*	6	12	1
not identifiable	15	31	19

178 strains were examined in more detail; the results of the examination are shown in table 14. The strains of *B. megaterium*, *B. cereus*, *B. cereus* var. *mycoides*, *B. subtilis*, *B. polymyxa*, *B. pantothenticus* and *B. sphaericus* were easily recognizable and were typical representatives of the respective species. For the remaining species the agreement with the standard descriptions in Bergey's Manual (7th ed. 1957) were less satisfactory in many cases, and the identification therefore difficult and uncertain. A considerable number of strains showed no resemblance at all to the species described in Bergey's Manual and are therefore tabulated as „not identifiable". Most of these strains were biochemically inactive with negative reactions in most of the tests.

It appears from the table that *B. cereus* is by far the most common of the *Bacillus* species, constituting c. 30 per cent of the strains examined. *B. cereus* var. *mycoides* has also been found in most of the soil samples, but much less frequently than in cultivated and fertilized soils. This is true to a still higher degree of *B. megaterium*, which is very uncommon in these soils, whereas both CONN (1948) and STOUT (1958, 1960) mention *B. cereus*, *B. cereus* var. *mycoides* and *B. megaterium* as the three most common *Bacillus* species.

B. sphaericus occurs also very frequently and has been found in practically all samples examined. The remaining species occur more sporadically. The examinations do not indicate a definite difference between mull and mor soils with regard to occurrence of the various *Bacillus* species.

Gram-positive and Gram-negative, pleomorphic rods

These two groups comprise strains with variable, irregular and often branched cells. They belong almost exclusively to the group of "coryneform" bacteria, comprising the genera *Corynebacterium*, *Arthrobacter* and *Nocardia*. True, acid-fast mycobacteria have not been observed.

37 strains of these pleomorphic rods have been examined more closely, and the details of this examination have been published elsewhere (JENSEN and FELUMB 1962). Only one of these strains could be identified as to species (*Arthrobacter citreus* Sachs). In the remaining strains not even the genera could be established with certainty. The boundaries are very indistinct between the three genera mentioned above, and many of the strains appeared to be transitional forms.

Streptomycetes

All strains classed with this group appeared as typical representatives of the genus *Streptomyces*. No other genera have been observed. A cursory examination indicated that the mull soils were richer in *Streptomyces* species than the mor soils. However, no attempts have been made at a closer identification of the strains.

"Others"

This last group consists of strains, which could not be classed with any of the preceding groups. It appears from table 15 that most of this group are yeasts and fungi. These two groups of organisms, however, will be treated in the next two papers of this series, and no further comments shall be given here.

Table 15

Distribution of 155 strains from the morphological group labelled "others"

	Gram-positive rods	Gram-positive cocci	*Vibrio* or *Spirillum*	Yeasts	Filamentous fungi
Beech mull . . .	13	4	4	16	20
Beech mor . . .	11	5	0	35	47

The bacteria of the last group have not been examined further because of their comparative insignificance. The rare occurrence of the true cocci is in good agreement with the findings of CONN (1948) and STOUT (1958, 1960) and confirm the assumption that the cocci do not belong to the soil microflora in the proper sense.

Discussion

The main purpose of this series of investigations has been to study quantitative and qualitative differences between the microflora found in various types of beech forest soils. In the preceding paper (JENSEN 1963) a clear quantitative difference was demonstrated between the bacterial flora of beech mull and that of beech mor, and the experiments recorded here show that there is also a clear qualitative difference.

In general, the investigations show that the bacterial flora of mull soils is richer in species and more varied than that of mor soils. This is particularly apparent from the distribution of the smaller groups: the Gram-negative, pigmented, non-sporeforming rods and the pleomorphic rods, which are uncommon or practically absent in mor soils. Apart from the sporeforming rods, however, this is true also of the larger groups, although not so readily demonstrable. The general impression after examination of the cultures belonging to these groups was that of a clearly richer variation between cultures from mull soils than between cultures from mor soils.

In the largest of the morphological groups, the Gram-negative, non-pigmented rods, the relative incidence varied within the same range for the two soil types. However, both nutritional classification and taxonomic examination have shown distinct differences in the composition of this group in mull and mor soils. It is probable that more detailed taxonomic studies may disclose many other differences, not manifested by the cursory examination made here. A thorough taxonomic treatment of this group is very desirable, but will entail great difficulties, due to the uncertain position of many genera and species within this group (cf. INGRAM and SHEWAN 1960, HOLDING 1960).

The group of sporeforming rods was the only group, where no substantial difference, either quantitative or qualitative, could be established between mull and mor soils. However, it is a well-known fact that the sporeforming bacteria are more ubiquitous and more independent of external factors than almost any other group of microorganisms.

When the composition of the bacterial flora of the forest soils studied here, is compared with the composition of the bacterial flora of cultivated and fertilized soils, as demonstrated by other investigators, great differences are observed.

In cultivated soils it is possible to distinguish between a zygomenous and an autochtonous microflora dependent on the supply of decomposable organic nutrients. In the autochtonous microflora, the small, Gram-positive, non-sporeforming, non-acid-fast rods, mainly belonging to the group of "coryneform" bacteria, outnumber all other types (TOPPING 1937, LOCHHEAD 1939). The zymogenous microflora found after addition of organic materials to the soil contains a much larger proportion of Gram-negative rods, particularly species of *Pseudomonas* and *Achromobacter*, and of sporeforming rods (CONN 1948, HOLDING 1960), and the same is true of the rhizosphere microflora.

In forest soils no such distinction is possible due to the continuous supply of organic materials in the form of plant residues throughout the year. Although most of the litter falls in the autumn, the intermixture of litter in the soil and its decomposition is extended throughout the entire year. The bacterial flora of forest soils, therefore, is mostly related to the zymogenous microflora of agricultural soils with a preponderance of Gram-negative rods, sporeformers and streptomycetes.

The nutritional grouping also shows that the very exacting bacteria, characteristic of the autochtonous flora of cultivated soils, are uncommon in forest soils. In these soils the distribution to the nutritional groups rather indicates a relationship to the rhizosphere microflora (cf. e. g. LOCHHEAD and THEXTON 1947) also readily explained by the continuous supply of organic nutrients.

Summary

A total of 4564 cultures (2051 from beech mull, 1921 from beech mor, 392 from mineral soils, 200 from spruce mor) were examined and classified on the basis of morphology (table 9). As a supplement, the biochemical activity of 372 strains was studied (table 3). 972 of the strains were classified on the basis of nutritional requirements (table 10). A closer taxonomic examination was made of a limited number of strains from some of the morphological groups.

The main results may be summarized briefly as follows:

About one third of the strains belonged to the largest of the morphological groups, the Gram-negative, non-pigmented rods (mainly species of *Pseudomonas* and *Achromobacter*). The relative incidence of this group was about the same in mull and mor, but there was a clear difference in the composition of this group in the two soil types.

The Gram-negative, pigmented rods (mainly flavobacteria) constituted about 10 per cent of the strains from mull soils, but only 2—3 per cent of the strains from mor soils.

The numbers of sporeforming bacteria varied very much, but the mean percentage was about the same in mull and mor (c. 15). No qualitative difference could be established.

The pleomorphic rods (mainly "coryneform" bacteria) were distinctly more common in mull soils (c. 6 per cent) than in mor soils (c. 2.5 per cent).

The number of streptomycetes varied greatly, but lay within about the same range in the two types of soil.

The general impression was that of a richer and much more varied bacterial flora in mull than in mor soils. Both the morphological and the nutritional classification indicate for both types of soil, relationships to the zymogenous microflora and the rhizosphere microflora of cultivated soils, rather than to the autochtonous microflora.

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